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ORIGINAL PAPER

Human bocavirus in children with acute lymphoblastic leukemia

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Abstract A new human parvovirus, human bocavirus, has recently been identified in respiratory secretions, feces and serum. It is associated with lower and most likely also upper respiratory tract infections. Most commonly reported symptoms are cough, rhinorrhea, expiratory wheezing and fever, and the virus is preferentially detected in young children. We report three children with acute lymphoblastic leukemia who had acute febrile episodes with concomitant detection of human bocavirus in their respiratory secretions. One of them had five consecutive febrile episodes during 6 months, all associated with the presence of human bocavirus at varying viral loads, suggesting prolonged shedding or reactivation of the virus.

Keywords Human bocavirus · Children · Cancer · Leukemia · Respiratory viruses

Abbreviations

HBoV	human bocavirus
RSV	respiratory syncytial virus
hMPV	human metapneumovirus
ALL	acute lymphoblastic leukemia
PCR	polymerase chain reaction
WBC	white blood cell count
CRP	C-reactive protein

Introduction

In children with cancer, one third of febrile episodes are caused by 14 respiratory viruses. The symptoms of respiratory virus infections can vary from mild to severe. Viral clearance may be delayed and prolonged viral shedding may occur [8, 18]. Respiratory syncytial virus (RSV), rhinovirus, adenovirus and parainfluenza viruses are the most frequently found viruses [4, 21]. Over the past 5 years, new viruses such as human metapneumovirus (hMPV), new coronaviruses (NL63 and HKU1) and human bocavirus (HBoV) have been identified as causing respiratory disease in humans [2, 10, 24]. The clinical impact of these new virus infections in immunocompromised children is not clear.

We report three children with acute lymphoblastic leukemia (ALL) suffering from symptoms of acute infection and positive for a new parvovirus, HBoV. One child had evidence of possible persistence or reactivation of HBoV infection, with repeated detection of HBoV in five consecutive febrile episodes during the anticancer treatment.

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Methods

Patients

We searched for respiratory viruses in children with acute leukemia. The survey was carried out from April 2000 through October 2005 at four Finnish university hospitals. A total of 51 children with acute leukemia participated in the study. The mean follow-up time was 1.5 years per patient (range 0.2 to 2.5 years, SD 0.6 years), and the mean age of the children was 5.9 years (range 0.4 to 15.3 years, SD 3.9 years). The study was approved by the Ethical Committee of the Medical faculties of Turku, Oulu, Kuopio and Helsinki Universities. Informed consent was obtained from the patients and their parents.

Methods

On admission or during hospital stay, a nasal swab was taken for viral studies each time the child had an axillary temperature of ≥38.0°C. The nasal swab sample was obtained through a nostril by inserting a sterile cotton swab to a depth of 2–3 cm and retracting it with rotating movements. The swab was then inserted into a vial containing viral transport medium (5% tryptose phosphate broth, 0.5% bovine serum albumin and antibiotics in phosphate-buffered saline) [9]. One aliquot of the sample was restored at −70°C. Virus culture was done by using the Ohio strain of HeLa cells and human foreskin fibroblasts according to routine procedure. Viral antigens for respiratory syncytial virus (RSV), adenovirus, parainfluenza virus types 1, 2 and 3, and influenza A and B viruses were detected using a time-resolved fluoroimmunoassay with monoclonal antibodies [1]. The details of the PCR assays used for rhinovirus, enteroviruses, human herpes virus 6 (HHV-6), coronavirus types OC43 and 229E 52 and human metapneumovirus (hMPV) have been reported earlier [1, 11]. The observations concerning these respiratory viruses will be reported separately.

Detection of human bocavirus

Human bocavirus was analyzed retrospectively from aliquots stored in a freezer at −70°C. DNA was extracted from a 220-μl aliquot of specimens using the NucliSens easyMag automated nucleic acid extractor (BioMèrieux, Boxtel, The Netherlands). The elution volume was 55 μl. PCR primers BoF (GGAAGAGACACTGGCAGACAA), BoR (GGGTGTTCCTGATGATATGAGC) and hydrolysis-probe BoP (Cy5- CTGCGGCTCCTGCTCCTGTGAT-BHQ2) targeting the NP-1 gene of HBoV have been described previously [1]. A cloned HBoV plasmid was used as a quantitative standard. PCR reactions were carried out in a 25 μl volume consisting of QuantiTect Probe PCR Master Mix (Qiagen, Hilden, Germany), 600 nM of each primer, and 100 nM of probe, and 5 μl extracted DNA. Amplifications were run on a Rotor-Gene 3000 (Corbett, Sydney, Australia) instrument with the following cycling conditions: 15 min at 95°C, 40 cycles of 15 s at 94°C and 60 s at 60°C. The plasmid standard could be detected at the level of a single copy per reaction. The lower limit for reproducible quantification was determinated to 500 genome equivalents (GE)/ml of sample material, and the reproducible detection limit was estimated to 200–500 GE/ml [10].

Results

A total of 125 nasal swab samples were investigated for HBoV by PCR and 7 were positive (5.6%).

Case reports

Case 1

A 4-year-old boy was diagnosed as having precursor-B-cell ALL in March 2001. Before hospitalization he had suffered for 3 days from fever (maximally 39.0°C) and cough. On admission, total white blood cell count (WBC) was 8.2×10^9/l (70% lymphocytes, 8% neutrophils and 19% blasts) and bone marrow aspiration revealed precursor-B-cell ALL. The serum C-reactive protein level was 104 mg/l. The treatment of leukemia was started on the second day of hospitalization. The patient had cough and rhinitis and otitis media, but no evidence of pneumonia on the chest radiograph. He was treated with broad-spectrum antibiotics. The blood culture remained negative. The PCR test for HBoV was positive in the nasal swab. The HBoV load was low, accounting for ≤500 copies per nasal swab sample. The stool sample was negative for HBoV. The 88 nasal swab samples were negative for 12 other respiratory viruses studied. The patient was discharged from the hospital 7 days after admission without symptoms of infection.

Case 2

A 3-year-old boy was diagnosed as having ALL in December 1999. In May 2000, he had a febrile episode while his leukemia treatment was in the consolidation phase and he was in remission. In addition to fever (38.6°C), he suffered from vomiting and diarrhea. The fever lasted for 1 day, and he was discharged after 2 days of hospitalization. His family members also had febrile gastroenteritis. On admission, the WBC count was 3.9 (75% neutrophils, 15%

lymphocytes, no blasts), and the serum C-reactive protein level was <1 mg/l. HBoV was detected from the nasal swab sample with a low number of copies of HBoV, ≤500 copies per nasal swab sample. The tests for the other 12 respiratory viruses remained negative. No stool sample was available.

Case 3

A 2-year-old boy was diagnosed to have a high risk precursor B-cell ALL in March 2000. He had 17 febrile infections during the anticancer treatment from March 2000 to March 2002. Between September 2000 and March 2001 he had five consecutive febrile infections with evidence of HBoV infection (Table 1). HBoV was detected from the nasal swab samples with varying numbers of copies of HboV. Fever was the only symptom of infection in three of five HboV-positive episodes. Concomitant other viruses were detected in only one episode: rhinovirus by PCR from the nasal swab sample and cytomegalovirus by antigen detection from the blood sample. In the other four episodes, the tests for the other 12 respiratory viruses remained negative. All five febrile episodes were treated with broad-spectrum antibiotics with full recovery.

Discussion

HBoV was first identified by Allander et al. in 2005 by large-scale molecular screening from the respiratory samples of children with lower respiratory tract disease [2]. Multiple studies worldwide have since confirmed the common presence of HBoV in children with both upper and lower respiratory disease [3, 6, 13–15, 25, 27]. HBoV may also be associated with diarrhea [3, 14, 15]. The occurrence of HBoV has been reported to be higher in children than in the adult population, varying from 5.6% to 19% in young children with respiratory disease [3, 13]. The association between HBoV and respiratory disease has been questioned because coinfections with other respiratory viruses occur in 33–83% of the HboV-positive cases and in most cases [1, 6]. Recently, we found HBoV in 19% of 259 children hospitalized due to acute expiratory wheezing. In 12 cases (5%) HBoV was the only virus detected and in 10 of these cases a high viral load suggested primary infection. HBoV DNA was identified also in the serum of these patients suggesting a systemic infection [1]. It is important that in four studies with totally 491 asymptomatic controls only 3 positive cases have been detected supporting the causative role of HBoV in symptomatic subjects [1, 6, 14, 17]. On the other hand, lifelong persistence and reactivation of other parvoviruses have been reported with and without symptomatic disease during immunosuppression [7, 20, 23].

We describe HBoV infection in children with cancer. Tests for 12 other respiratory viruses remained negative in six out of seven reported febrile episodes. These tests found other concomitant respiratory viruses in 30 of 49 cases positive for human bocavirus in our previous study [10]. The occurrence of HBoV in febrile children with leukemia in our study was 5%. Our patients had symptoms of febrile respiratory disease and febrile gastroenteritis. They all recovered from infection within days without any need for intensive care. The causative role of HBoV, however, for febrile infection in our immunosuppressed patients is not clear. Most episodes were associated with HboV at low copy numbers, which we have suggested to present asymptomatic shedding in immunocompetent children. Our third case is, to our knowledge, the first reported patient with repetitive detection of HBoV showing prolonged shedding or reactivation over a 5-month period, thus supporting the hypothesis that HBoV may persist for a long time after primary infection [1]. It is not certain that the same HBoV strain was detected all the time, because no sequence studies were carried out. The genetic variability of HBoV is on the other hand very low [5, 22]. One may speculate that the high viral load detected in two febrile episodes indicates that HBoV could be a causative agent of

Table 1 The details of the child with leukemia and five consecutive febrile episodes with evidence of HBoV infection

Date	Treatment at presentation	Clinical features	HboV copy number/sample	Concomitant microbes	Serum CRP level (mg/l)	WBC ($\times 10^9$/l)
Sep 2000	Consolidation	Fever 1 day, rhinitis	81,000	None	85	2.3
Nov 2000	Consolidation	Fever 3 days	<500	None	75	1.3
Dec 2000	Maintenance	Fever 2 days	<500	None	14	1.4
Feb 2001	Maintenance	Fever 3 days, stomatitis, wheezy bronchitis	1,400	Rhinovirus, cytomegalovirus	161	0.1
Mar 2001	Maintenance	Fever 3 days	100,000	None	116	0.7

the febrile episodes, while the HBoV at low genome copy numbers may represent carriage. In only one episode were other viruses detected. Rhinovirus persistence and chronic infection in lungs has recently been demonstrated in lung transplant patients with a fatal outcome [12].

Previously, one adult cancer patient with severe atypical pneumonia associated with HBoV detection has been reported [19]. This patient had symptoms of fever and cough, and a computer tomography scan of the lungs showed bilateral reticulonodular infiltrations. HBoV was detected from BAL samples, while all bacterial and fungal tests as well as tests for other respiratory viruses remained negative. Her symptoms subsided within days, and no mechanical ventilation was needed. Recently, HBoV detection in other adult and pediatric immunocompromised patients with previous organ transplants or patients with HIV infection have been reported from large respiratory disease surveys [3, 19, 26].

Currently, a great number of respiratory viruses are to be searched for in febrile infections. In some cases, a virus infection may explain a poor response to antibiotic treatment, and specific antiviral therapy may be available. On the other hand, respiratory virus infections may also pave the way to invasive bacterial infections, leading to even more severe morbidity. Searching for respiratory viruses should be a part of clinical practice in febrile children with cancer. Whether detection of respiratory viruses in these patients reflects primary infection, reinfection, persistence or reactivation deserves further studies.

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